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Straightforward chemo-enzymatic synthesis of new aminocyclitols, analogues of valioline and their evaluation as glycosidase inhibitors

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Abstract—An efficient fructose-1,6-bisphosphate aldolase mediated synthesis of new aminocyclitol analogues of valioline is described. The one-pot process where four stereocentres are created involves the formation of two carbon–carbon bonds. One is catalysed by the aldolase, coupling dihydroxyacetone phosphate to nitrobutyraldehydes. The other is the result of a highly stereoselective intramolecular Henry reaction occurring on the intermediate nitroketones. Depending on the configuration of the hydroxyl which is α to the nitro group, two series of configuration are accessible. The lipase resolution of the nitroalcohol ketal, precursor of the nitroaldehyde, is presented. The inhibition properties of the aminocyclitols obtained after the reduction of the nitro group are evaluated towards five commercial glycosidases.

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1. Introduction

Glycosidases are enzymes widely distributed in micro-organisms, plants and animals. They selectively hydrolyse glycosidic bonds and play important roles in crucial biological pathways such as polysaccharide and glycoconjugate anabolism and catabolism,¹ cellular recognition² and eukaryotic glycoprotein processing.³ Glycosidases are also involved in a variety of metabolic disorders and diseases such as diabetes, viral or bacterial infections and cancer formation. Consequently, their inhibitors have many potential applications⁴ such as antidiabetic,⁵ antiviral (VIH, influenza)⁶ and anticancer⁷ drugs.

The design and synthesis of glycosidase inhibitors are mainly focused on mimicking the transition state (TS) that occurs in an enzymatic glycoside hydrolysis.⁸ A partial positive charge develops at the anomeric carbon and at the endocyclic oxygen (Fig. 1) or at the exocyclic oxygen.

Natural aminocyclitols (aminocarbascars) such as valioline 1, validamine 2, valienamine 3 (Fig. 1) and their ana-

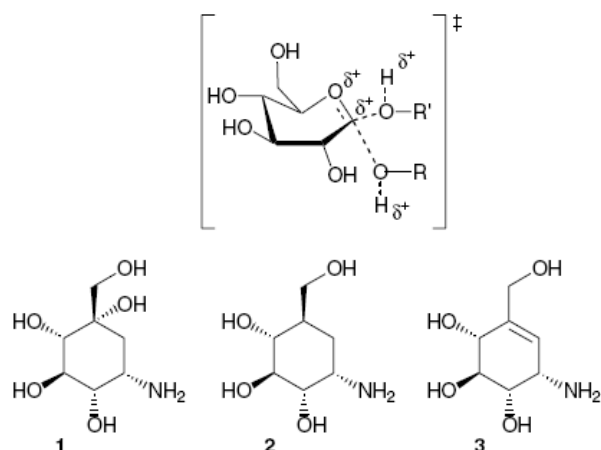


Figure 1. Transition state model and natural aminocyclitols.

logues can be effective specific inhibitors of glycosidases involved in intestinal degradation of carbohydrates. They are supposed to be partially protonated in the active site at physiological pH, mimicking the TS where the positive charge is located on the exocyclic oxygen.

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As a consequence, a large variety of synthetic approaches have been used to develop this class of compounds, ranging from chemical to enzymatic methods, and employing starting materials ranging essentially from sugars to natural carbocyclic compounds.⁹

Recently we reported the first fructose-1,6-diphosphate aldolase mediated synthesis of aminocyclohexitol **5** (Fig. 2).¹⁰ To access the target compound, our strategy used the condensation of dihydroxyacetonephosphate (DHAP) catalysed by the aldolase (RAMA) on 4-nitroaldehyde **4**.

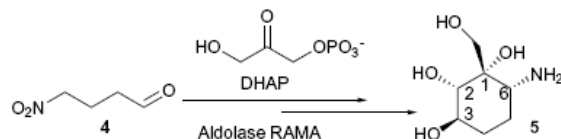
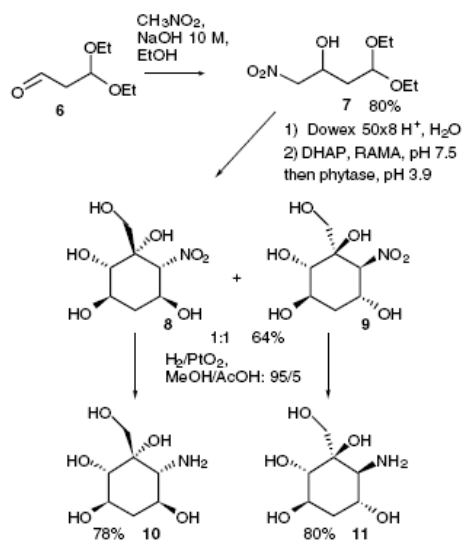


Figure 2. Aldolase-mediated synthesis of aminocyclitol.

The enzyme controlled the configuration of two stereocentres (2 and 3), forming the C2–C3 bond. The second carbon–carbon bond formation (1 and 6) was the result of a highly stereoselective intramolecular Henry reaction. In such a process, an enantiomerically pure nitrocyclitol bearing four asymmetric centres was formed. In this report, following this new methodology, we document the synthesis of two nitrocyclitols and their corresponding aminocyclitols with an additional hydroxyl group on C5. The kinetic resolution of the precursor via a lipase is presented. We furthermore report activities of the latter as glycosidase inhibitors.

2. Results and discussion

Aldehyde diethyl acetal **6** (Scheme 1) was prepared by ozonolysis of the corresponding alkene¹¹ previously ob-



Scheme 1. Synthesis of aminocyclitols.

tained using the procedure of Hoaglin et al.¹² The nitroaldolisation (Henry reaction) between compound **6** and nitromethane gave nitroalcohol **7** in an 80% yield. This compound has been succinctly described by Yanovskaya et al.,¹³ NMR and MS data have not been provided. We fully characterised it to complete its analytical data. Acid-mediated hydrolysis of the ketal furnished an intermediate aldehyde (quantitative from TLC), which was used directly in the aldolisation reaction after pH adjustment to 7.5. Rabbit muscle aldolase (RAMA) and DHAP¹⁴ were added to the reaction mixture. After the DHAP was consumed (checked by enzymatic assay),¹⁵ the pH was adjusted to 3.9 and phytase¹⁰ added to hydrolyse the phosphate group. Two new nitrocyclitols **8** and **9** were isolated in a 64% yield (from **7**) in a 1:1 ratio. They were separated by flash chromatography.

Their absolute configurations (Fig. 3) were determined from NMR data (NOEs and coupling constants), based on the aldolase stereoselectivity, which induces the formation of stereocentre 2 with an (*S*)-configuration. Interestingly, the configuration of the alcohol functionality on **7** influenced nitrocyclisation. When the alcohol was (*S*), nitrocyclitol **8**, possessing the same (1*S*,6*R*)-configurations as compound **5** (Fig. 2) was isolated. When the alcohol was *R*, the configuration 1*R*,6*S* was obtained for compound **9**. We can reasonably assume that the reaction performed in water and at rt was under thermodynamic control. The two nitrocyclitols are the more stable isomers. In both cases, the hydroxymethyl, the nitro and the hydroxyl at position 5 are equatorial. For compound **9**, two hydroxyl groups (on carbons 2 and 3) are in an axial position. The reduction of the nitro group of **8** and **9** was then performed over PtO₂ under 50 psi of hydrogen. The two aminocyclitols **10** and **11** were isolated with the same 76% yield.

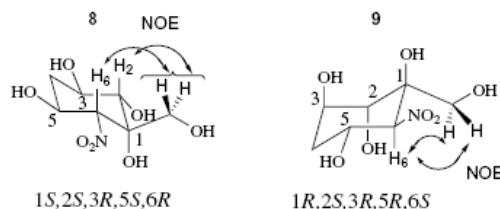


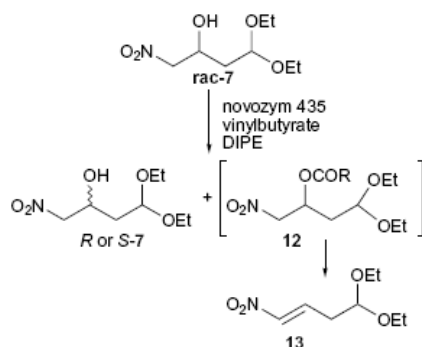
Figure 3. NOE's, configurations and major conformations of compounds **8** and **9**.

In order to improve yields and avoid the chromatographic separation of nitrocyclitols **8** and **9**, we decided to study the lipase catalysed kinetic resolution of alcohol **7**. We expected to resolve **7** by direct enzymatic acylation of the hydroxy group. All of the results are presented in the table (Table 1).

Three enzymes (entries 1–3) were tested and lipase B from *Candida antarctica* showed the best activity. Alcohol **7** was not a substrate of lipase PS and PC (amano AK and PS, respectively). Different solvents were screened (entries 4–6) and diisopropylether (DIPE) gave the best results. Ester **12** underwent spontaneous elimination, giving a large amount of alkene **13** (Scheme 2).

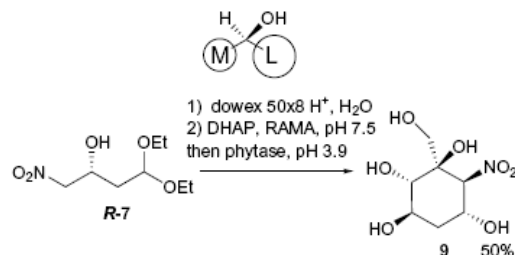
Table 1. Kinetic resolution of **7** at rt

Entry	Enzyme	Molarity	Acyl donor (equiv)	Solvent	<i>t</i> (d)	ee ^a (%)	<i>c</i> ^a (%)	<i>E</i>
1	CAL-B ^b	0.483	Vinylacetate (1.2)	TBME	6	55	60	3.6
2	PSL ^b	0.483	Vinylacetate (1.2)	TBME	—	—	—	—
3	PCL ^b	0.483	Vinylacetate (1.2)	TBME	—	—	—	—
4	CAL-B ^b	0.483	Vinylacetate (1.2)	DIPE	10	86	55	15
5	CAL-B ^b	0.483	Vinylacetate	Vinylacetate	10	25	30	—
6	CAL-B ^b	0.483	Vinylacetate (1.2)	CH ₂ Cl ₂	—	—	—	—
7	CAL-B ^b	0.483	Succinic anhydride (1.2)	DIPE	—	—	—	—
8	CAL-B ^b	0.483	Vinylbutyrate (1.2)	DIPE	7	97	52	75
9	CAL-B ^b	0.483	Vinylpalmitate (1.2)	DIPE	7	9	24	—
10	CAL-B ^c	0.120	Vinylbutyrate (2)	DIPE	2	92	50	78

^a Determined by HPLC using 1,3,5-trimethoxybenzene as an internal standard.^b Ratio in weight enzyme:alcohol (1:2.5).^c Ratio in weight enzyme:alcohol (1:4).**Scheme 2.** Lipase resolution of alcohol **7**.

This type of result was also observed by Sheldon et al. with an analogous compound possessing a phenyl group at the same place as our ketal functionality.¹⁶ Ester **12** was observable by HPLC, while the reaction medium was kept under anhydrous conditions. Even after several attempts to purify it with different workups, we always ended with alkene **13** as the major product of the reaction. At this point, we turned our attention to the influence of the acyl donor, trying to select a less good leaving group by increasing the alkyl chain length (entries 7–9). No reaction was observed when succinic anhydride was used as the acyl donor. This phenomenon was also observed by Sheldon et al.¹⁶ with the phenyl analogue cited above. The best result, with a largely improved *E*, was obtained with vinylbutyrate (entry 8). On a preparative scale (entry 10), we recovered alcohol **7** in a 92% ee and 50% yield. Finally, we were never able to avoid the formation of alkene **13**. From the steric model for the preferentially converted enantiomer of secondary alcohols by CAL-B, we expected to prepare alcohol **7** with an (*R*)-configuration (**Scheme 3**). This was confirmed by chemical correlation using the aldolase to convert **7** into a nitrocyclitol. Cyclitol **9** was isolated in a 50% yield (**Scheme 3**), which confirmed the *R* configuration for alcohol **7**.

Finally, we comment on the inhibitory activities of the amines prepared in this study against five commercial glycosidases. Amines **10** and **11** were not active against the α -glycosidase tested (see Experimental). On the contrary, they moderately and selectively inhibited the β -glycosidases (β -glucosidase and β -galactosidase). The *K_i* found for com-

**Scheme 3.** Steric model for the preferentially converted enantiomer of secondary alcohols by *Candida antarctica* lipase and chemical correlation.

pound **10** were 0.117 ± 0.03 mM and 1.46 mM, and for compound **11** 2.27 and 2.62 mM, respectively for β -glu and β -gal. Compound **10**, mimicking the stereochemistry of glucose more closely was the best inhibitor for β -glucosidase. More generally, the selectivity for β -glycosidases could be due to the equatorial position of the amine group. On the contrary in valiolamine, a natural inhibitor of α -glucosidases, this amino group is in the axial position.

3. Conclusions

As a conclusion, we carried out another efficient synthesis to prepare two new nitrocyclitols and aminocyclitols in a highly stereoselective one-pot/two enzyme process. This illustrates the versatility of our strategy to synthesise aminocyclitols in just a few steps, reducing considerably the laborious protection–deprotection steps always found when sugars are used as starting material. Enzymatic resolution of the alcohol precursor showed that the elimination reaction was difficult to avoid, and allowed us to successfully prepare the enantiomerically pure ketal precursor with *R* configuration. The amines were found to be moderate and selective inhibitors towards β -glycosidases.

4. Experimental

4.1. General procedures

All the reagents and solvents were of commercial quality and were purchased from chemical companies. For chro-

matographic purification, technical grade solvents were distilled prior to use. Merck 60 F254 silica gel TLC plates and Merck 60/230-400 and 60/40-63 mesh silica gel for column chromatography were used. Visualisation of the developed chromatogram was performed by oxidative staining by either KMnO_4 - NaHCO_3 solution or vanillin solution. Optical rotations, reported in $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$, were measured with a Jasco Dip-370 polarimeter. IR spectra were recorded on an FT IR Perkin Elmer 881 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer in CDCl_3 , D_2O and CD_3OD . J values are given in Hz and δ in ppm, referenced to the internal solvent signals for ^1H and ^{13}C . Fructose-1,6-diphosphate aldolase from rabbit muscle (RAMA; EC 4.1.2.13, suspension in ammonium sulfate) and phytase from *Aspergillus ficuum* (EC 3.1.3.8, crude) were from Sigma. *C. Antartica* lipase type B (CAL-B, Novozyme 435) was acquired from Sigma. High performance liquid chromatography (HPLC) analyses were carried out in a Waters 590 chromatograph UV detector at 210 nm using a Daicel Chiracel OD column (25 cm \times 4.6 mm ID) (hexane/isopropanol 98/2, 0.7 mL/min). New cyclitols synthesised were more or less hygroscopic. Final characterisation of most of these compounds was therefore done by high resolution mass spectra (HRMS), recorded by the Centre Régional de Mesures Physiques de Clermont-Fd, France.

4.2. Experimental procedures

4.2.1. 4,4-Diethoxy-1-nitrobutan-2-ol 7. To a solution of aldehyde **6**^{11,12} (2 g, 13.7 mmol, 1 equiv) in EtOH (10 mL) was added nitromethane (750 μL , 13.7 mmol, 1 equiv) followed by NaOH 10 N (1.37 mL, 13.7 mmol, 1 equiv). The solution was stirred at 0 °C for 45 min. The mixture was neutralised with AcOH (785 μL , 13.7 mmol, 1 equiv) and diluted with 10 mL water, extracted with 3 \times 100 mL ether. The combined organic phase was dried over MgSO_4 , filtered and concentrated in vacuo. The residue was purified by chromatography on silica gel, eluting with cyclohexane/AcOEt (6/4), to afford the target compound as a slightly yellow oil. R_f = 0.42 cyclohexane/AcOEt (6/4). ^1H NMR (400 MHz, CDCl_3): δ 4.74 (t, 1H, J = 5 Hz); 4.57 (m, 1H); 4.45 (dd, 2H, J = 2.3, 7 Hz); 3.72 (m, 2H); 3.58 (m, 1H); 3.55 (m, 2H); 1.88 (m, 2H); 1.22 (m, 6H). ^{13}C NMR (100 MHz, CDCl_3): δ 101.3; 80.4; 65.8; 62.8; 62.5; 37.2; 15.2. IR (thin film) ν (cm^{-1}) 3435; 1550; 1376; 1125. SM (IC): m/z 206; 190; 162; 144; 103.

4.2.2. (1S,2S,3R,5S,6R)-1-Hydroxymethyl-6-nitrocyclohexane-1,2,3,5-tetraol 8 and (1R,2S,3R,5R,6S)-1-hydroxymethyl-6-nitrocyclohexane-1,2,3,5-tetraol 9. To a solution of **7** (400 mg, 2.65 mmol) in 5 mL water was added a cation exchange resin (Dowex 50x8, H^+ form, 1.5 g). The suspension was stirred at 45 °C for 2.5 h (quantitative by TLC). The resin was filtered off, the pH was adjusted to 7.5 with 1 M NaOH. To this solution was added DHAP (3.62 mL, 1.34 mmol, 1 equiv) followed by 30 mL water, and the pH was adjusted to 7.5 with 1 M NaOH. The mixture was bubbled with Ar and previously centrifuged aldolase (60 U) was added. After stirring 24 h at rt, the mixture was washed with 3 \times 20 mL AcOEt. The water phase pH

was adjusted to 3.9 with 1 M HCl and phytase (92 U) was added. The resulting solution was stirred at rt for 24 h, then concentrated under vacuum. The residue was purified by chromatography on silica gel, eluting with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (9/1 then 8/2), to give the target compounds **8** (96 mg, 32%) and **9** (95 mg, 32%) as brown solids.

Data for **8**, R_f = 0.18 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$: 85/15). F = 136 °C. $[\alpha]_D^{25}$ = +27 (c 2.67, CH_3OH). ^1H NMR (400 MHz, CD_3OD): δ 4.65 (d, 1H, J = 10.2 Hz); 4.5 (ddd, 1H, J = 10.2, 5.1, 12 Hz); 3.8 (ddd, 1H, J = 9.3, 12, 4.7 Hz); 3.78 (d, 1H, J = 11 Hz); 3.4 (d, 1H, J = 9.3 Hz); 3.25 (d, 1H, J = 11 Hz); 2.27 (ddd, 1H, J = 4.7, 5.1, 12 Hz); 1.43 (ddd, 1H, J = 12, 12.2, 12 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 93.3; 76.6; 75; 69; 66.7; 62; 39.1. IR (KBr) ν (cm^{-1}) 3480; 1545; 1380; 1063. SM (IC): m/z : 246 ($\text{M}+\text{Na}$); 228. Anal. found, C, 37.01; H, 5.88; N, 5.95. $\text{C}_7\text{H}_{13}\text{NO}_7$ requires C, 37.67; H, 5.87; N, 6.28.

Data for **9**, R_f = 0.36 ($\text{CH}_2\text{Cl}_2/\text{MeOH}$: 85/15). F = 153 °C. $[\alpha]_D^{25}$ = -33.3 (c 4.25, CH_3OH). ^1H NMR (400 MHz, CD_3OD): δ 4.67 (ddd, 1H, J = 11, 4, 11 Hz); 4.63 (d, 1H, J = 11 Hz); 4.03 (ddd, 1H, J = 3, 3, 3 Hz); 3.96 (d, 1H, J = 3 Hz); 3.77 (d, 1H, J = 11.5 Hz); 3.42 (d, 1H, J = 11.5 Hz); 2.15 (ddd, 1H, J = 3, 3, 13.5 Hz); 1.98 (ddd, 1H, J = 3, 11, 13.5 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 93.5; 78.4; 72; 70.3; 65.4; 64.9; 36.4. IR (KBr) ν (cm^{-1}) 3480; 1544; 1378; 1063. SM (IC): m/z : 246 ($\text{M}+\text{Na}$); 228. Anal. found, C, 37.66; H, 5.87; N, 6.28. $\text{C}_7\text{H}_{13}\text{NO}_7$ requires C, 37.67; H, 5.87; N, 6.28.

4.2.3. (1S,2S,3R,5S,6R)-6-Amino-1-hydroxymethylcyclohexane-1,2,3,5-tetraol 10. To a solution of nitrocyclitol **8** (80 mg, 0.32 mmol) in MeOH/AcOH (95/5) (40 mL) was added PtO_2 (20 mg). The mixture was submitted to 50 psi of H_2 in a Parr apparatus. After stirring for 48 h at rt, the catalyst was removed by ultrafiltration and washed with MeOH. The filtrate was concentrated under vacuum, and the crude product was purified by cation exchange chromatography (Dowex® 50WX8, 200–400 mesh, H^+ form) eluted with 1 M NH_4OH . Compound **10** was obtained as a white solid in 78% yield (48 mg). R_f = 0.22 ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$: 8/1/1). F = 101 °C. $[\alpha]_D^{25}$ = -7.9 (c 1.1, H_2O). ^1H NMR (400 MHz, CD_3OD): δ 4.65 (d, 1H, J = 10.2); 4.5 (ddd, 1H, J = 10.2, 5.1, 11.7, 5.1 Hz); 3.8 (ddd, 1H, J = 9.3, 12, 4.7 Hz); 3.78 (d, 1H, J = 11 Hz); 3.4 (d, 1H, J = 9.4); 3.25 (d, 1H, J = 11); 2.27 (ddd, 1H, J = 4.7, 5.1, 12 Hz); 1.43 (ddd, 1H, J = 12, 12.2, 12 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 74.4; 70.1; 69.9; 65.7; 63.6; 57.7; 33.9. IR (KBr) ν (cm^{-1}) 3414; 1110. SM (IC): m/z : 193.

4.2.4. (1R,2S,3R,5R,6S)-6-Amino-1-hydroxymethylcyclohexane-1,2,3,5-tetraol 11. Compound **11** was isolated as a white solid following the same protocol as described above, in 80% yield (49 mg). R_f = 0.32 ($\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$: 8/1/1). F = 76 °C. $[\alpha]_D^{25}$ = +6.6 (c 1.2, H_2O). ^1H NMR (400 MHz, CD_3OD): δ 4.00 (ddd, 1H, J = 3, 3, 3 Hz); 3.92 (ddd, 1H, J = 11, 4, 11 Hz); 3.75 (d, 1H, J = 3 Hz); 3.73 (d, 1H, J = 11.5 Hz); 3.62 (d, 1H, J = 11.5 Hz); 3.16 (d, 1H, J = 10 Hz); 2.07 (ddd, 1H, J = 3, 3, 13.5 Hz); 1.83 (ddd, 1H, J = 3, 11, 13.5 Hz). ^{13}C NMR

(100 MHz, CD₃OD): δ 74.8 70.1; 69.9; 65.5; 64.2; 57.3; 33.9. IR (KBr) ν (cm⁻¹) 3414; 1110. SM (IC): m/z : 193.

4.2.5. Lipase kinetic resolution of 7 by transesterification

4.2.5.1. General. In a typical procedure for analytical study, the corresponding acyl donor was added to a suspension of 7 and CAL-B in DIPE (or other solvent cited in the table) and the mixture was shaken at RT following the progress of the reaction by chiral HPLC (Chiracel OD) and 1,3,5-trimethoxybenzene as an internal standard. After removal of the enzyme by filtration and evaporation of the solvent, the residue was purified by flash chromatography (cyclohexane/AcOEt (8/2)) to give alcohol (R)-7 and alkene 13.

4.2.5.2. For preparative scale. (R)-4,4-Diethoxy-1-nitrobutan-2-ol 7. In the procedure described above, racemic alcohol 7 (0.5 g, 2.41 mmol, 1 equiv) and CAL-B (2 g) in DIPE (20 mL) and vinylbutyrate (630 μ L, 4.96 mmol, 2.06 equiv) as acyl donor were used. The alcohol (R)-7 (460 mg) was obtained in a 49% yield, and 92% ee after a flash chromatography purification. $[\alpha]_D^{23} = -10.4$ (c 1.22, CHCl₃). Spectral data were identical to the racemic sample.

4,4-Diethoxy-1-nitrobut-1-ene 13. In the above procedure, alkene 13 (190 mg) was obtained in a 41% yield.

$R_f = 0.54$ (cyclohexane/AcOEt: 7/3). ¹H NMR (400 MHz, CD₃OD): δ 7.17 (m, 1H); 6.96 (d, 1H, $J = 14$ Hz); 4.53 (t, 2H, $J = 5.3$ Hz); 3.62 (qd, 2H, $J = 7.1$ Hz); 3.43 (qd, 2H, $J = 7.1$ Hz); 2.51 (dd, 2H, $J = 5.3, 5.4$ Hz); 1.16 (t, 6H, $J = 7.2$ Hz). ¹³C NMR (100 MHz, CD₃OD): δ 141.1; 137.4; 100.5; 62.0; 33.2; 15.2. IR (thin film) ν (cm⁻¹) 1652; 1528; 1349; 1122; 1061. SM (IC): m/z : 189.

4.2.6. Chemical correlation. Nitrocyclitol 9 was obtained from (R)-7 following the protocol described above (97 mg, 51%) as a brown solid.

4.3. Inhibition studies

α -glucosidase from rice, α -glucosidase from baker's yeast, β -glucosidase from almond, α -galactosidase from green coffee beans, β -galactosidase from *Aspergillus oryzae*, α -mannosidase from jack beans and all substrates (4- or 2-nitrophenyl α - or β -glycopyranosides) were purchased from Sigma. Assays were run at 25 °C in a phosphate buffer (25 mM) at pH 6.8 using the corresponding 4-nitrophenyl-glycoside in a total volume of 1 mL. The potential inhibitors were tested at a final concentration of 1 mM and the amount of enzyme of each assay was adjusted so that the system would give the initial rate. After two periods (5 min and 30 min) of incubation of the enzyme in the presence of the tested molecule, the substrate was added and the optical absorbance was followed at 400 nm. The initial rate was determined, compared to the one obtained without the tested molecule, and the percentage of inhibition was calculated. When the percentage of inhibition was higher than 33%, the K_i was determined

according to the Hanes–Woolf method. Four substrate concentrations (0.04–2.5 mM) and four inhibitor concentrations (0.005–0.8 mM) were chosen. The K_i was then calculated from the Michaelis–Menten (K_M and four K'_M) constants obtained in the presence or absence of inhibitor. When the percentage of inhibition was between 33% and 10%, the K_i was determined with the following equation $K_i = [I]/(K'_M/K_M - 1)$, using only one K'_M value.

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